C3 cont

(c) differentiating the precursor cells in a second culture medium that is substantially free of said growth factor to produce oligodendrocytes, and

(d) causing the oligodendrocytes to come into contact with a demyelinated axon to effect remyelination of said demyelinated axon.

C4

25. A method of producing glial cells from a neural stem [cells] cell, the progeny of said neural stem [cells] cell being capable of differentiating into neurons, astrocytes and oligodendrocytes, said method comprising the steps of:

(a) [isolating] dissociating said neural stem [cells] cell from [a] donor tissue,

(b) proliferating the [isolated] dissociated neural stem [cells] cell in a first culture medium containing a growth factor to produce precursor cells, and

(c) differentiating the precursor cells in a second culture medium that is substantially free of said growth factor to obtain glial cells.

Please add the following claim:

C5 36. The method of claim 25 wherein said first culture medium is defined.

REMARKS

As recommended by the Examiner, this application has been reviewed for errors. Accordingly, the specification has been amended to correct minor grammatical errors.

Claims 1, 7 and 25 are amended in response to the Examiner's rejection under § 112, first paragraph as discussed in more detail below.

Claims 4 and 5 are amended to further distinguish the claimed methods from the methods disclosed in the cited references. Support for these amendments exists in the specification as discussed in detail below.

New claim 36 is added to recite an embodiment of the invention wherein a defined culture medium is used to proliferate the stem cells. This claim is supported by the specification at page 16, lines 18-29.

<u>Provisional Obviousness-type Double Patenting Rejection</u>

Applicants note that the rejection of the claims as being unpatentable over the claims of copending application serial no. 08/010,829 in view of Cepko is <u>provisional</u>, i.e. is dependent upon whether subject matter is allowed in the present application or co-pending application serial no. 08/010,829. Accordingly, Applicants will address this rejection when there is an indication of allowable subject matter in either this or the copending application.

Applicants also wish to draw the Examiner's attention to co-pending application U.S. Ser. No. 08/481,893, filed June 7, 1995, which discloses and claims subject matter disclosed and claimed in the present application. Eleven other copies of the '893 application were also filed on June 7, 1995. If necessary, the Examiner can telephone the undersigned to obtain the serial numbers of these other related applications.

<u>Rejection under 35 U.S.C. § 101</u>

The Examiner maintains the rejection of claims 1 to 17 under § 101. The Examiner states that the Declaration of Dr. Hammang does not overcome the rejection because the amended claims require "that the isolated neural stem cells be capable of differentiating into neurons, astrocytes and oligodendrocytes and the declaration states that the transplanted cells differentiated into oligodendrocytes." However, from the combined teachings of the specification and the declaration of Dr. Hammang, it should be readily apparent that while at least some of the neural stem cell progeny that were injected into myelin deficient rats differentiated into oligodendrocytes to effect remyelination, the neural stem cell progeny are indeed ***capable*** of also differentiating into astrocytes and neurons under certain culture conditions.

In paragraph 4 of the declaration, Dr. Hammang refers to the cells cultured in the experiments as "multi-potential EGF-responsive stem cells". The term "multi-potential" refers to an undifferentiated cell that is capable of producing multiple types of differentiated progeny. Dr. Hammang states the source and culture conditions of the stem cells: cells

obtained from the striata of E14-15 rats and mice propagated in a defined, serum-free medium containing 20 ng/ml EGF. Turning to the specification, page 8, line 5 to page 9, line 11, discloses that these EGF-responsive cells will, upon removal of the mitogen (e.g. EGF) adhere to a substrate and differentiate into neurons and glia. Thus, the neural stem cells used in the experiments described by Dr. Hammang are indeed capable of differentiating into neurons, astrocytes and oligodendrocytes. However, rather than being subject to conditions that induced these cells to differentiate into neurons, the undifferentiated cells were implanted adjacent areas of demyelinated neurons *in vivo*, where many of the cells appropriately differentiated into oligodendrocytes to remyelinate the neurons. The mechanism by which the transplanted cells "knew" to differentiate into oligodendrocytes as opposed to neurons is not entirely understood. However, an understanding of the mechanism of action is not a requirement of patentability.

If requested by the Examiner, another declaration of Dr. Hammang can be prepared that states that cells obtained and cultured using the same methods as in the remyelination experiments (and described in the specification), were allowed to proliferate and differentiate *in vitro* into neurons, astrocytes and oligodendrocytes. However, it is believed that in view of the above explanation, the Examiner will consider this to be unnecessary, cumulative information.

Rejections under 35 U.S.C. § 112

Claims 1 to 17 remain rejected under § 112, first paragraph. Regarding claims 1 and 7, the Examiner considers that the usefulness of remyelination of axons in vitro is not apparent. However, one of ordinary skill in the art would recognize that the methods of the present application could be used in vitro in conjunction with drug screening applications to determine whether certain drugs, growth factors, and other biological agents enhance or inhibit remyelination. U.S. Ser. No. 08/270,412, filed July 5, 1994, which is a continuation of U.S. Ser. No. 07/726,812, filed July 8, 1991, of which the present application is a continuation-in-part application and which is incorporated by reference into the present

application, discusses the utility and application of precursor cells in drug screening assays. Accordingly, it is believed that the rejection of claims 1-17 should be withdrawn.

The Examiner objects to the specification and to claims 1-17 and 25-35 for failing to disclose "isolation" of a stem cell population. By the term "isolated", it is not meant that a pure population of stem cells is obtained. Rather, it is meant that a suspension of (at least some) individual cells is obtained so that, under appropriate culture conditions, the cells can be induced to proliferate. Accordingly, the claims are amended to clarify that the neural stem cells are dissociated from donor tissue. Support for this amendment is in the paragraph bridging pages 9 and 10 of the specification.

In view of the amendment to claims 1, 7, and 25, it is believed that the second rejection under § 112, first paragraph is overcome.

Rejections under 35 U.S.C. § 102

Claims 25-28, and 30-34 remain rejected under § 102 (b) as being anticipated by Hunter et al. The goal of Hunter et al. was to increase the number of O-2A progenitor cells which give rise to oligodendrocytes and type II astrocytes. The method described by Hunter and Bottenstein selectively expanded the O-2A progenitor cell population, resulting in 75-90% of the cells in their cultures becoming O-2A progenitors. O-2A progenitor cells are not the same as the multipotent neural stem cells defined by the present application. The differences between "progenitor" and "stem" cells is discussed on page 5 of the previous response (mailed May 31, 1994). However, in view of comments made by the Examiner in an office action mailed September 28, 1995, in related application U.S. Ser. No. 08/376,062, Applicants believe further clarification is necessary. In the office action of the related application, the Examiner stated that "Cattaneo discloses that the cells were nestin+ and since it is known in the art that nestin+ positive cells give rise to neurons and glial cells, Cattaneo does disclose methods of culturing multipotent stem cells." (p 8, line 4 of office action). Nestin is an intermediate filament protein found in many types of undifferentiated CNS cells, including both progenitor cells and stem cells. Therefore, it is not a marker that can distinguish stem

cells from progenitor cells.

Attached as Appendix A is a diagram that illustrates the *in vitro* proliferation of multipotent neural stem cells which is also summarized in the specification on page 8, line 12 to page 9, line 29. A neural stem cell, in the presence of a proliferation-inducing growth factor (e.g. EGF) and appropriate culture conditions (A), proliferates to form a sphere of proliferating, undifferentiated neural cells, referred to as a "neurosphere". The neurosphere is comprised of daughter stem cells and daughter progenitor cells. The cells of a neurosphere are the progeny of a single stem cell, and thus are clonally-derived. The neurosphere can be passaged to induce further stem cell proliferation by dissociating the neurosphere (B) to form a cell suspension and resuspending the cells in fresh medium containing a growth factor. In the continued presence of a growth factor, stem cells within the cell suspension proliferate further (C) to result in the formation of new neurospheres. Alternatively, the dissociated cells of the neurosphere can be cultured in conditions that induce differentiation of progenitor cells (e.g. by plating the cells on a fixed substrate such as poly-L-lysine and/or removal of the proliferation-inducing growth factor). Under differentiation-inducing conditions, progenitor cells may undergo limited proliferation (D) before undergoing terminal differentiation (E) into neurons and/or glial cells (oligodendrocytes and type I and II astrocytes). As disclosed in the Hunter reference, O-2A progenitor cells are bipotential, giving rise only to oligodendrocytes and type II astrocytes. Referring to Appendix A, the Hunter *et al.* method occurs at steps D and E (proliferation and terminal differentiation of progenitor cells). The progeny of the O-2A progenitor cells are not capable of differentiating into neurons or type I astrocytes.

The "neurospheres" formed using Applicants' methods should not be confused with the aggregates formed using the methods of Hunter *et al.*. As illustrated by step A of the diagram of Appendix A, ***a neurosphere is formed by the <u>clonal</u> expansion of a single cell.*** In contrast, the Hunter culture method uses a high concentration of heparin which causes the cells that normally adhere to the substratum to stick to each other and form floating aggregates (see Hunter p. 236, column 2, last full paragraph).

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The Examiner's basis for the rejection is that because Hunter et al. started with brain tissue obtained from neonatal rats, the primary cultures prepared inherently contained stem cells since stem cells were not removed from the cultures. The Examiner states "that production of progeny cells is an inherent property of stem cells", therefore, Hunter et al. "inherently discloses stem cells capable of differentiating into neurons, astrocytes and oligodendrocytes." For a proper anticipation rejection on the grounds that a prior art reference inherently anticipates a claimed invention, "the Examiner must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic ***necessarily*** flows from the teachings of the applied prior art." [*Ex parte Levy*, 17 USPQ2d, 1461, 1464 (Bd. Pat. Appeals & Int'f 1990); emphasis in original].

Step (b) of claim 25 requires that the neural stem cells are proliferated. While neural stem cells may have been present in the primary cultures of Hunter et al., this is not sufficient to maintain an anticipation rejection based on the doctrine of inherency because it does not necessarily follow that the stem cells, if present, were induced to proliferate under the conditions of Hunter *et al.* Once a stem cell is placed in a culture medium *in vitro*, several fates of the cell are possible depending on the culture conditions. The stem cell may: 1) die, 2) proliferate to produce progeny cells, or 3) survive the culture conditions, but not proliferate (i.e. remain quiescent). The culture conditions used by Hunter *et al.* differ significantly from Applicants' conditions. Some of the differences with the Hunter *et al.* culture method include the use of an undefined cell-conditioned culture medium, poly-D-lysine coated flasks, and high concentrations of heparin in the initial culture medium which alters the adhesive properties of the cells.

The attached declaration of Dr. Reynolds details experiments that were performed in order to determine whether the culture conditions of Hunter *et al.* induce the proliferation of stem cells. In summary, primary neurospheres, known to contain neural stem cells and generated using the methods described in Example 1 of the specification, were harvested. The cells were dissociated and passaged by culturing them at 6.3×10^6 cells per T25 flask using one of the following conditions:

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1. Hormone medium and 20 ng/ml EGF, no substrate (Applicants' passaging culture conditions, known to induce neural stem cell proliferation).

2. 67% O3 medium plus 33% B104 conditioned medium, using a poly-D-lysine and fibronectin substrate (Hunter and Bottenstein's passaging culture conditions).

After 4 days in culture, small, attached neurospheres were present in the cultures grown under condition 1. The presence of the neurospheres indicated that neural stem cells present in the flask were proliferating. The flasks prepared according to condition 2 contained no neurospheres. Instead, attached cells, resembling multipolar oligodendrocytes and astrocytes were observed. This indicates that stem cells obtained from neural tissue, remain quiescent in the culture conditions of Hunter and Bottenstein. In other words, <u>the culture conditions of Hunter and Bottenstein do not induce stem cell proliferation</u>. Proliferation is defined as "a rapid and repeated production of new parts or of buds or offspring (as in a mass of cells by a rapid succession of cell divisions or in a coral by the production of buds in quick succession)" [Webster's Third New International Dictionary (1981)]. If the conditions of Hunter and Bottenstein did induce stem cell proliferation, neurospheres indicating the clonal proliferation of neural stem cells, should have been observed. By comparison, 87.5% of the neurospheres formed using applicants' methods generated new spheres upon dissociation and reculturing. On average, 69 neurospheres were generated from each dissociated neurosphere, indicating that neural stem cells rapidly proliferate using applicants' methods.

The method of claim 36 is further distinguishable from the methods of Hunter *et al.* Claim 36 requires that the culture medium used to proliferate the neural stem cells is defined. Support for this claim is noted above. The culture medium used by Hunter *et al.* contains cell conditioned medium and thus, is not defined (i.e. the entire molecular make-up of the medium is not known).

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<u>Rejections under 35 U.S.C. §103</u>

The Examiner maintains the rejection from the previous office action that claim 29 is unpatentable over Hunter in view of Morrison. Claim 29 requires the proliferation of neural stem cells in a culture medium containing epidermal growth factor and the differentiation of the progeny of the neural stem cells (i.e. precursor cells) in a culture medium that <u>does not</u> contain EGF.

The Examiner states that "Hunter was cited to disclose the proliferation of neural stem cells." As detailed above and in the attached declaration of Dr. Reynolds, the methods of Hunter *et al.* do not result in the proliferation of neural stem cells. Furthermore, Hunter *et al.* teach away from using EGF to proliferate undifferentiated neural cells by demonstrating that "...epidermal growth factor (EGF) had no growth-promoting effects at the tested doses (p. 242, col. 1) when tested on their population of enriched 0-2A progenitor cells.

The Examiner cites Morrison as disclosing that "EGF stimulates the proliferation and differentiation of glial cells." As noted in Applicants' previous response, this cite from the Morrison paper is based on the teaching of Almazan (Reference F of form PTO 1449 mailed May 31, 1994). Almazan cites other references that show that EGF induces proliferation of <u>differentiated glial cells</u> in culture (p. 257, col. 2). It was well known at the time of the Almazan publication that, unlike neurons that cease proliferating after differentiation, differentiated glial cells *in vitro* are able to proliferate after differentiation and, the presence of EGF can induce glial cell differentiation. The Almazan paper teaches that aggregates of fetal rat brain tissue demonstrate increased <u>differentiation</u> of the cells in the presence of EGF, but not proliferation (p. 263, concluding paragraph).

In summary, Hunter, Morrison and Almazan, taken together or individually, fail to teach or suggest a method for the proliferation of <u>undifferentiated</u> neural stem cells in the presence of EGF, followed by the differentiation of stem cell progeny in the absence of EGF as required by claim 29. Hunter teaches that EGF does not induce proliferation of O-2A progenitor cells; Morrison teaches that EGF promotes survival and stimulates process outgrowth of differentiated neurons; and Almazan teaches that EGF increases differentiation

of neonatal rat brain cells (and cites other publications that demonstrate EGF-induced proliferation of already differentiated glial cells).

The Examiner maintains the rejection of claims 1, 3-6 and 35 under § 103 as being unpatentable over Boyles taken with Hunter, Gage and Masters. The Examiner states that "since Hunter and Masters started with brain tissue... the cell cultures obtained therefrom would necessarily contain the 'neural stem cell' since neither Hunter nor Masters removed the 'neural stem cells' from the cell cultures". As discussed above, applicants' claimed methods require the proliferation of multipotent neural stem cells in the presence of a growth factor. It is not enough that the starting material merely contains a multipotent neural stem cell. Applicants have already demonstrated that the method of Hunter *et al.* does not induce stem cell proliferation. While the method of Masters *et al.* starts with tissue that may contain multipotent stem cells, their method requires the isolation of oligodendrocyte progenitor cells from the starting material before culturing the isolated oligodendrocyte progenitors in a growth factor-containing culture medium (see page 119, 10th line in methods section; p. 123, 5th line from bottom; and reference #13 cited on p. 130). Accordingly, neither Hunter *et al.* nor Masters *et al.* teach or suggest the proliferation of multipotent neural stem cells in a culture medium containing a growth factor.

Applicants do not understand why the Boyles *et al.* reference is cited. It merely discloses the fact that a variety of types of apolipoproteins are present in nervous tissue where they play a role in allowing lipids to cross cell membranes, and that their levels increase in crushed sciatic (peripheral) nerves. Boyles *et al* teach various sources of apolipoproteins including plasma HDL (ubiquitous component of blood plasma) and neurons, in addition to glial cells (see p. 17812, col. 1). ApoD (which the examiner makes reference to) is produced by the crushed nerves (see the abstract, line 10 from the bottom). Thus, to follow the Examiner's argument, if a supply of apolipoproteins was required for remyelination to take place, then astrocytes and oligodendrocytes would not be necessary for remyelination as this molecule is available from several other sources. However, the principal reason why oligodendrocytes are transplanted is not specifically to provide apoD, or any other similar

molecule in order to facilitate the repair process, but to form a physical cellular layer (the myelin sheath) around the axons. Most physiology text books have illustrations of the myelination process and show the physical relationship of the oligodendrocytes (or Schwann cells in the peripheral nervous system) to axons.

In any event, neither the Masters *et al.* nor the Hunter and Bottenstein references disclose the proliferation of neural stem cells. Accordingly, the Examiner's rejection of claims 1, 3-6 and 35 should be overcome on this basis alone.

With regards to claims 5 and 6 the Examiner states that "Gage clearly suggests transplantation of autologous stem cells for therapeutic purposes". However, the Gage patent fails to teach or suggest using the progeny of ***neural*** stem cells derived from a patient's own tissue for transplantation purposes. Gage clearly indicates that (at the time the Gage application was filed) there was a "paucity of replicating non-transformed cell culture systems" which could be used for neural transplantation purposes (see col. 14, lines 61-66). Thus, the Gage method uses fibroblasts (non-neural cells) for transplantation.

Claim 5 is amended to define the recipient of the autologous transplantation method as a juvenile or adult. Support for the amendment is in the specification at page 9, lines 31-33, wherein the use of adult neural tissue is disclosed. Support also exists in U.S. Ser. No. 08/270,412, filed July 5, 1994 (incorporated by reference into the present application) at page 12, lines 20-25 which discloses the use of cells proliferated *in vitro* obtained from juvenile and adult tissue for autologous transplantation. Applicants' discovery of a neural stem cell in the adult mammalian brain, which has the ability to proliferate *in vitro* and differentiate into neurons, astrocytes, and oligodendrocytes, was surprising to highly skilled neurobiologists because, at the time of the invention, it was believed that neural stem cells simply do not exist in the adult central nervous system. Attached as Appendix B is an article from page one of The New York Times, (March 27, 1992), which demonstrates that researchers in the field of neurobiology were of the belief, at the time of the invention, that neural stem cells were not found in the adult mammalian CNS. Applicants respectfully request the Examiner to read this short article, as it may help put into perspective how Applicants' technology

(characterized as "pioneering" by Dr. McKay of M.I.T.) differs from the prior art. Note the comment made by Dr. Emmanuel DiCicco-Bloom: "It wasn't thought possible that you would find this (stem cells that can proliferate and produce neurons) in the mature mammalian brain". The Court of Appeals for the Federal Circuit has averred that "expressions of disbelief by experts constitute strong evidence of nonobviousness [Environmental Designs, Ltd. v. Union Oil Co. of Calif (CAFC 1983) 218 USPQ 865, 868]. Accordingly, the comments made by various researchers in the field of neuroscience which are quoted in this article provide compelling secondary evidence of the unobvious nature of the claimed invention.

Regarding claim 4, in response to Applicants' arguments (presented in response to the previous office action) that a "neurosphere" is clonally derived, whereas the aggregates of Hunter *et al.* are normally adherent cells that have been forced to stick together due to high concentrations of heparin in the culture medium, the Examiner states that "neither claim 1 nor 4 has the phrase 'clonally derived neurospheres' and therefore Applicants' arguments are not persuasive." Claim 4 is amended to define the neurospheres as "clonally-derived." As detailed in the specification, a neurosphere forms when a single neural stem cell proliferates to form a cluster of cells (this is also illustrated in Appendix A at step "A" of the diagram). Therefore, one of ordinary skill in the art of culturing cells would appreciate that the cells of the neurosphere are "clonal" in nature because they are the progeny of a single cell. The proliferation of neural stem cells and the formation of neurospheres is described on page 8, line 12, to page 9, line 5, and on page 10, line 13-24.

The Examiner states that "a careful reading of the specification shows... that neurospheres, contrary to Applicants' arguments... contain some cells which are nestin (+)... comprised of cells which are stem cells and/or progenitor cells and may or may not include differentiated cells." (Emphasis in original). Applicants are not certain why the Examiner makes this statement, but speculate that, because more than one phenotype may be expressed, the Examiner doubts the assertion that the cells of the neurosphere are "clonally-derived". Indeed, all of the cells of the neurosphere are initially immunoreactive for nestin and do not

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express any of the markers of differentiated cells (see p. 8, lines 12-28). However, if the neurosphere is allowed to remain in culture without reinitiation of proliferation (e.g. passaging the spheres to a new culture and dissociation of the spheres to form a single cell suspension in which stem cells further proliferate to form new spheres), over a period of some time some of the cells within the neurosphere begin to differentiate and are no longer nestin (+). This is described in the sentence bridging pages 41 and 42 of the application incorporated by reference, U.S. Ser. No. 08/270,412. The term "clonally-derived" does not mean that the cells have the same phenotype. Instead, it is an art-recognized term that means that the cells are the progeny of a single parent cell. Therefore, applicants' claimed methods clearly differ from the methods of Hunter *et al.* that result in the formation of aggregates of unrelated cells.

In view of the foregoing, it is believed that the rejection of claims 1, 3-6 and 35 under § 103 as being unpatentable over Boyles taken with Hunter, Gage and Masters, should be withdrawn.

The Examiner maintains the rejection of claim 2 under §103 as being unpatentable over Boyles taken with Hunter, Masters, Gage and Morrison. The comments made above with respect to what these references teach are incorporated here by reference. The Examiner states that "Applicants have argued that the method of claim 2 calls for the proliferation of neural stem cells. However, contrary to such arguments, claim 2 merely requires the use of EGF." It is not understood what the Examiner means by this. Claim 2 is dependent on claim 1, and thus contains all the limitations of claim 1, including the limitation that stem cells are proliferated in a culture medium containing a growth factor [see step (b) of claim 1]. Claim 2 requires that the growth factor is EGF. It is reasserted that Morrison *et al.* teach that epidermal growth factor promotes the proliferation of <u>glial cells</u> (i.e. cells which have already differentiated into glial cells), not the proliferation of neural stem cells as required by claim 2. Accordingly, the rejection of claim 2 should be withdrawn.

The Examiner maintains the rejection of claims 7, 8, 10-13 and 15-17 under §103 as being unpatentable over Boyles taken with Hunter, Masters and Gage. The Examiner states that "since Hunter discloses preparation of a primary neural cell culture wherein the cells

were derived from the rat brain, Hunter discloses isolating neural stem cells from the tissue of a donor, lacking evidence to the contrary." The method of claim 7 does not merely require the presence of a neural stem cell, but also requires its proliferation. Not only does the Hunter *et al.* reference fail to teach or suggest the proliferation of neural stem cells using their culture methods, the attached declaration of Dr. Reynolds demonstrates that the culture conditions of Hunter *et al.* do not promote neural stem cell proliferation.

The Examiner states that "regarding claims 15-17, Applicants have argued that Gage does not teach or suggest the transplantation of oligodendrocytes...". The Examiner rebuts this argument by stating that "claims 15-17 do not require transplantation of oligodendrocytes." Applicants do not understand the Examiner's statement. Claims 15-17 depend upon claim 7 which in step (d), clearly requires contacting oligodendrocytes with a demyelinated axon. The Examiner made a similar comment with respect to claim 2 (i.e. that the claim does not require the proliferation of stem cells -- a feature clearly set forth in claim 1 from which claim 2 depends). Unless applicants have misinterpreted the Examiner's comments, it appears that the Examiner has disregarded applicants' arguments set forth in the previous response based on a misunderstanding of the limitations of the dependent claims. Section 608.01(n) of the MPEP states:

> Examiners are reminded that a dependent claim is directed to a combination including everything recited in the base claim ***and*** what is recited in the dependent claim. It is this combination that must be compared with the prior art, exactly as if it were presented as one independent claim. (Emphasis in original).

Accordingly, applicants respectfully request the Examiner to reconsider the arguments presented in the response mailed May 31, 1994, with respect to claims 15-17 and the teachings of the Gage patent (as well as the arguments made in response to the rejection of claim 2).

The Examiner maintains the rejection of claim 9 under §103 as being unpatentable over Boyles taken with Hunter, Masters, Gage and Morrison. The Examiner states that "Applicants have argued that Morrison does not teach the proliferation and differentiation of

glial cells". However, applicants agree that the prior art teaches the use of EGF to induce (already differentiated) glial cells to proliferate and further differentiate. What applicants have argued is that Morrison does not teach the use of EGF to induce proliferation of neural stem cells (i.e. undifferentiated cells). Claim 9 requires the use of epidermal growth factor for the proliferation of neural stem cells (not glial cells). The combination of references cited by the Examiner fail to teach or suggest this. Instead, the references teach the use of EGF for the proliferation and differentiation of already differentiated glial cells and further teach that EGF does not induce proliferation of undifferentiated cells (see Hunter *et al.*).

Also with regards to claim 9, the Examiner states that "Hunter was not cited to teach stimulation of proliferation using EGF". This comment was apparently made in response to applicants' argument that "Hunter *et al.* found no growth-promoting effects when EGF was added to their cell cultures." Thus, it seems that the Examiner's obviousness rejection is based on selectively picking and choosing various features of the cited references without looking at what the references teach as a whole. It is error to find obviousness "in considering the references in less than their entireties, i.e., in disregarding disclosures in the references that diverge from and teach away from the invention at hand" [W.L. Gore & Assoc. v. Garlock, Inc., 220 USPQ 303, 311 (Fed. Cir. 1983)]. See also In re Rosenberger et al., where Appellants "invented a method... in the face of art which strongly suggests that such a method would produce unacceptable results." The Court held that "this is the very antithesis of obviousness" [(CCPA 1967) 156 USPQ 24, 26]. Accordingly, the Examiner cannot ignore the teaching of the Hunter *et al.* reference that EGF does not induce the proliferation of undifferentiated cells.

New Grounds of Rejection under §103

Claim 14 stands rejected under §103 as being unpatentable over Boyles, Hunter, Gage, Masters and Freshney. As explained above, none of the references cited by the Examiner disclose a method for the proliferation of neural stem cells. Therefore, cloning the cells of the cited references would not produce the invention as claimed in claim 14.

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Claims 31-34 stand rejected under §103 as being unpatentable over Hunter or Almazan *et al.* taken with Freshney. The Examiner states that "Hunter discloses producing glial cells comprising isolating neural stem cells from a donor (Abstract)." However, Hunter only discloses the proliferation of O-2A glial progenitor cells. As explained above, a neural stem cell is not the same as an O-2A progenitor cell. The declaration of Dr. Reynolds, demonstrates that neural stem cells do not proliferate using the methods of Hunter *et al.* The Examiner also states that "Hunter discloses differentiating the precursor cells in a second culture medium which is substantially free of said growth factor to obtain glial cells" because "a culture medium containing 33% B104 CM is considered to be substantially free of the growth factor." However, a culture medium containing B104 CM would contain growth factors (see second sentence of the Hunter & Bottenstein abstract).

The Examiner also states that "Almazan discloses that neurons and glial cells within the aggregates proliferate and differentiate in culture." The Examiner seems to equate the term "clonally-derived neurosphere" (i.e. a cluster of cells derived from a single proliferating stem cell) with the "aggregates" of cells described in the Hunter *et al.* and Almazan *et al.* papers. Almazan discloses preparing "aggregating cell cultures" according to the methods of Honegger and Richelson ("Growth and differentiation of aggregating fetal brain cells in a serum free defined medium" *Nature (Lond.)*, Vol. 282 (1979) 305-307). A copy of this paper is attached as Appendix C. As can be seen, their method causes "aggregation" of cells, rather than the formation of clonally derived clusters of cells. They refer to the "re-aggregation" of cells which had been dissociated and then resuspended in culture medium (p. 305, col. 1, 1st ¶). Thus, using their method, the separated cells came together (i.e. re-aggregated) to form clusters of unrelated cells.

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CONCLUSION

In view of the amendments to the claims, and for the above reasons, it is believed that the present application is in condition for allowance. The claimed methods are readily distinguishable from the methods of the prior art. The Examiner is encouraged to telephone the undersigned with any questions there may be regarding any of the discussions or information submitted with this response or if any pages of this facsimile are not legible and need to be resent.

Respectfully submitted,

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